On-line Stereochemical Process Monitoring by Molecular Rotational Resonance Spectroscopy

Justin L. Neill[1][*], Yuan Yang[2], Matt T. Muckle[1], Roger L. Reynolds[1], Luca Evangelisti[3], Reilly E. Sonstrom[4], Brooks H. Pate[4], and B. Frank Gupton[2]

[1]BrightSpec, Inc., 770 Harris St. Suite 104b, Charlottesville, VA 22903
[2]Department of Chemical and Life Science Engineering, Virginia Commonwealth University, Richmond, VA
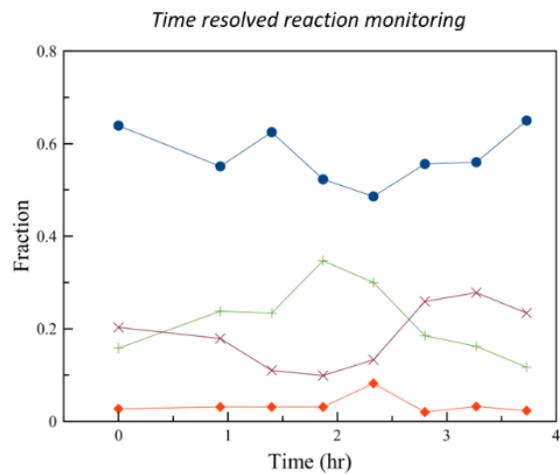[3]Dipartimento di Chimica "G. Ciamician" dell'Universita, via Selmi 2, I-40126, Bologna, Italy
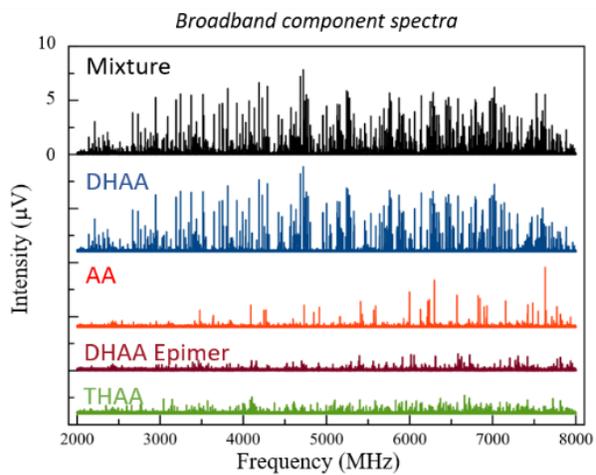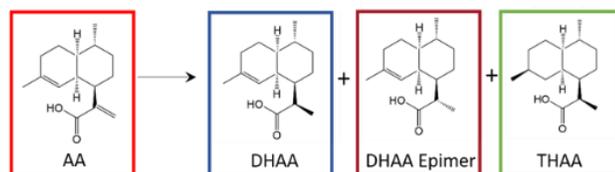[4]Department of Chemistry, University of Virginia, McCormick Rd, Charlottesville, VA 22904

[*]Corresponding author: Justin L. Neill: justin.neill@brightspec.com, Tel: +1 434 202 2391.

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Abstract

  A molecular rotational resonance (MRR) spectrometer designed to monitor the product composition of an asymmetric continuous flow reaction on-line is presented. The MRR technique is highly sensitive to small changes in molecular structure, and as such is capable of rapidly quantifying isomers as well as other impurities in a complex mixture, without chromatographic separation or chemometrics. The spectrometer in this study operates by automatically drawing a portion of the reaction solution into a reservoir, volatizing it by heating, and measuring the highly resolved MRR spectra of each of the components of interest in order to determine their relative quantity in the mixture. The reaction under study was the hydrogenation of artemisinic acid, an intermediate step in the semi-synthesis of the anti-malarial drug artemisinin. Four analytes were characterized in each measurement: the starting material, the product, a diastereomer of the product, and an overreduction byproduct that was not directly quantifiable by either HPLC or NMR methods. The MRR instrument has a measurement cycle time of approximately 17 minutes for this analysis, and can run for several hours without any user interaction.

Introduction

  On-line reaction monitoring techniques are extremely valuable for enabling rapid process understanding, development, and optimization. In particular, quickly characterizing the yield, specificity, and impurities generated in a chemical reaction is very important. In contrast to off-line analyses, which require the user to manually sample, transport, measure, and read results, on-line instrumentation can run continuously during a chemical reaction, archiving data and alerting the process chemist when an out-of-specification result is obtained. As a result, extensive efforts have been undertaken to apply the suite of analytical chemistry instrumentation to on-line reaction monitoring applications, including FT-IR[1-2] and NMR[3-6] spectroscopies, mass spectrometry,[7] and chromatography.[8-12] Nevertheless, significant challenges remain in characterizing all of the relevant species present in a chemical process, additional development is still needed. The United States Food and Drug Administration (FDA), through its Process

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Analytical Technology (PAT) initiative, encourages these developments to further its goals of improved process control and real-time testing for quality assurance.[13]

One critical parameter in pharmaceutical manufacturing that is often not monitored online, due to the absence of suitable methods, is the chiral purity of the product being synthesized. The number of new small-molecule drugs with at least one chiral center has increased considerably over the past few decades, with most of these now manufactured as single enantiomers.[14,15] In addition, many drugs contain multiple chiral centers, leading to additional stereoisomers known as diastereomers. A variety of chirally-sensitive chromatography and spectroscopy techniques have been developed for resolution of enantiomers, while for diastereomers – which are chemically similar but have different three-dimensional structures – techniques with high structural selectivity are needed.[16-18] Techniques for distinguishing each of the possible stereoisomers of a molecule – both enantiomers and diastereomers – are necessary in order to obtain full process analysis and control in the synthesis of chiral drugs.

Only a few studies demonstrating on-line reaction monitoring of stereoisomers have been published to date. Several studies have shown the capability for vibrational circular dichroism (VCD) to monitor the enantiomeric excess of a chemical reaction[19-21], while fast chiral chromatography methods have also been demonstrated.[22,23] In this paper, we present the application of molecular rotational resonance (MRR) spectroscopy for on-line reaction monitoring of a pharmaceutical process. MRR, also widely known as microwave or rotational spectroscopy, is extremely sensitive to small differences in molecular structure, and as such is well suited for the differentiation of isomers.[24] Due to the high spectral resolution of MRR, a mixture of components, including isomers (regioisomers, diastereomers, *cis-trans* isomers, or isotopologues), can be resolved without the need for separation or chemometrics. Additionally,

while not demonstrated directly in this work, recent advances have enabled the resolution of enantiomers by MRR using two methods – chiral tagging and three-wave mixing.[25] Chiral tagging can be performed to determine enantiomeric purity on the instrument described on this paper without modification. This work is motivated by recent advances in continuous manufacturing technology in pharmaceutical processing,[26-28] and seeks to demonstrate the feasibility of MRR as a technique for directly measuring stereoisomeric purity, as well as other impurities in the reaction, at relevant points along small-molecule synthetic processes.

The reaction under study is the Ru/C catalyzed hydrogenation of artemisinic acid (AA), which can be produced from genetically engineered yeast,[29] to dihydroartemisinic acid (DHAA) in a trickle-bed flow reactor. DHAA can thereafter be converted into artemisinin through photo-oxidation[30]; the diastereomeric selectivity of the hydrogenation step is critical in achieving high yield of the final product, artemisinin.[31-33]

Experimental Methods

Broadband MRR spectroscopy for component identification

In order to determine the MRR transition frequencies for each component in the hydrogenation reaction, an off-line 2-8 GHz broadband MRR spectrometer was employed. The design of this instrument has been described elsewhere.[34,35] A total of four samples were measured: commercially obtained crystalline high-purity samples of artemisinic acid (AA) and dihydroartemisinic acid (DHAA); an intentionally over-reduced sample in order to produce the overreduction byproduct, tetrahydroartemisinic acid (THAA); and finally, a crude reaction mixture containing AA, DHAA, THAA, and a diastereomeric impurity of DHAA (epimerized at

the chiral center created by the hydrogenation). The latter two samples were provided as oils (approximately 70 mg each).

Solid samples were transferred directly into a 0.9 mm Parker Hannefin Series 9 pinhole nozzle, modified to incorporate a sample reservoir that can be heated to volatilize liquid and solid samples. Oil samples were first dissolved in ethanol (at a concentration of approximately 100 mg/mL) in order to allow for their transfer by syringe into the nozzles. The sample was split among 3 nozzles, oriented in parallel, run simultaneously in order to increase measurement sensitivity. Samples were heated to approximately 160°C to produce suitable vapor pressure (~1 Torr) for analysis. High-grade (>99.999%) neon was used as the carrier gas.

Process monitoring MRR spectrometer

The process MRR spectrometer employed in this study is presented in Figure 1. The instrument design is based on the Fabry-Perot microwave cavity spectrometer originally developed by Balle and Flygare[36] and later miniaturized by Suenram *et al.*[37] The dimensions of this spectrometer are the same as those described by Suenram *et al*. A pair of spherical aluminum mirrors, held under high vacuum (~10^{-6} Torr), are coupled to a microwave generator that produces pulses with a frequency that is resonant with a mode of the cavity. The vacuum is generated by a 6-inch diffusion pump with a rotary vane backing pump. Volatilized molecular samples are injected into the cavity region in synchronization with the microwave pulses. When the microwave pulse is resonant with a molecular rotational transition of a component in the sample, the sample emits a free induction decay (FID) signal, which is coupled out of the cavity, digitized, and Fourier transformed to yield the MRR spectrum.

In comparison to the broadband spectrometer, this cavity-enhanced MRR spectrometer is faster and consumes significantly less sample (by a factor of approximately 100) to achieve a given sensitivity level. However, this instrument can only detect a narrow bandwidth in one measurement - in most cases, a single molecular transition around one of the resonant cavity modes - due to the high cavity finesse. One of the mirrors in the cavity spectrometer is motor tunable, so that its position can be changed in about 5 seconds to measure a different transition within the spectrometer bandwidth (9-18 GHz). Transitions from each of the components of interest are measured sequentially during each measurement cycle.

Figure 1: Photograph of the MRR spectrometer used for the measurements presented in this study.

The amount of sample required for analysis by MRR - both in the initial broadband characterization, and the process measurements - depends on the level of sensitivity needed. This is because the sample, after it is volatilized and injected into the MRR spectrometer, is evacuated through the system's vacuum system. To this point, we have not undertaken any attempts to recover samples. In the case of the reaction studied here, the strongest components in the mixture

can be observed in a broadband measurement with 10 mg or less of sample – or with approximately 100 μg of sample in a targeted analysis (where the MRR spectrum of the analytes is already known). However, since the signal-to-noise ratio of the MRR spectrum depends on the measurement time, if lower-level impurities are desired, more sample and more measurement time are needed. The amount of sample required to reach a given sensitivity level also depends on the molecule's intrinsic MRR spectral strength – which depends primarily on the permanent dipole moment and the molecular weight of each analyte.

Sampling interface

A schematic and photographs of the interface between the continuous flow reactor and the process MRR spectrometer are presented in Figure 2. The output of the flow reactor is directed into a gas-liquid flow separator, which was designed to give the MRR spectrometer access to a constantly refreshing reservoir of fresh reaction mixture for analysis while allowing the hydrogen gas in the line to escape. As more of the product solution comes out of the reactor, the solution in the reservoir overflows and exits the separator. We estimate that this reservoir, with a volume of about 2 mL, turns over approximately every 2 minutes under typical reaction flow rates.

When the MRR spectrometer is ready to begin a new analysis, a solenoid dosing pump is used to deliver a fixed volume of the reaction mixture through a valving manifold and into a heated reservoir nozzle. This nozzle is the same design as for the broadband MRR measurements described in the previous section, except it was fitted with two tube connections on the sides of the nozzle to allow direct introduction of solutions. This nozzle is inserted into the back of the stationary cavity mirror. The reservoir is first heated to a temperature slightly below the boiling

point of the solvent, with the MRR spectrometer tuned to a solvent transition to monitor the remaining solvent concentration in the sample. For this reaction, with ethanol (bp 78.4°C) as the solvent, a temperature of 75°C was chosen. Once the solvent is nearly evaporated, the reservoir temperature is increased to 160°C to volatilize the analytes. The selected transitions of each species of interest are then measured, followed by a brief period of reservoir evacuation at a higher temperature (in this case, 190°C) to evacuate any remaining sample. Once the reservoir is clean, the nozzle is cooled back to the solvent volatilization temperature to receive the next sample. The total cycle time to analyze the 4 observed species in the DHAA reaction, including sample delivery, measurement, and cleaning, is about 17 minutes with the current system. The amount of sample consumed per measurement is 0.99 mg (330 μL of solution mixture with a total analyte concentration of 3 mg/mL). The reservoir has a usable volume of approximately 500 μL; however, methods can be developed to accommodate larger injection volumes if necessary (if, for example, the analyte concentration in the solution is very low).
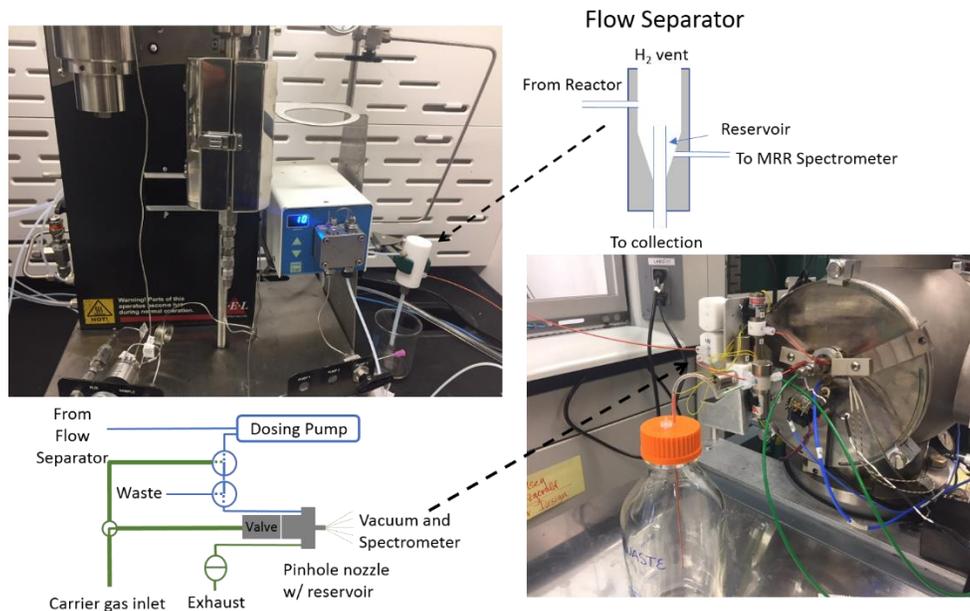
Figure 2: Images of the interface between the continuous hydrogenation process and the MRR spectrometer for reaction monitoring. Top left: The flow catalysis reactor. A flow separator is used to collect a reservoir of liquid and allow the hydrogen gas to escape. Top right: An internal cutaway drawing of the flow separator. Bottom left: Schematic of the valving manifold to transfer the reaction solution into the nozzle for volatilization. Bottom right: photograph of the valving manifold mounted to the spectrometer. (The blue cables observed in this figure are the microwave signal input and output.)

Results and Discussion

Broadband spectroscopy of reaction mixture

In Figure 3, the broadband MRR spectrum of the crude DHAA reaction mixture is presented. Each component in the species is identified by modeling its spectrum to a rotational Hamiltonian, where the primary derived parameters are the three molecular rotational constants (proportional to the inverse of the moments of inertia along the three principal axes of the molecule). Each component in this mixture, has two low-energy conformational isomers, differing by a 180° rotation of the carboxylic acid group; each of these is detected as a unique pattern in the spectrum. Compounds are identified by comparison of the experimental rotational constants of each fit to calculated values from electronic structure theory (see the Supporting Information for further description). Because the spectrum consists of approximately 100,000 spectral resolution elements, and each analyte spectrum usually consists of between 100-200

detectable transitions, overlaps between the spectral transitions of different species are extremely rare, as Figure 3 shows. Therefore, we can monitor the concentration of each species without interference by monitoring a single line known to be assigned to that species. A more complete analysis of these spectra, including structural parameters of these species as derived from the molecular moments-of-inertia, will be presented in a future paper.

We note that the bandwidth of the process monitoring MRR spectrometer (9-18 GHz) did not overlap with the broadband MRR spectrometer used in this study (2-8 GHz). We used the fits from the broadband spectrometer to predict the frequencies and intensities of strong transitions in the bandwidth of the process spectrometer, which were confirmed by subsequent measurement of trial reaction mixture samples.
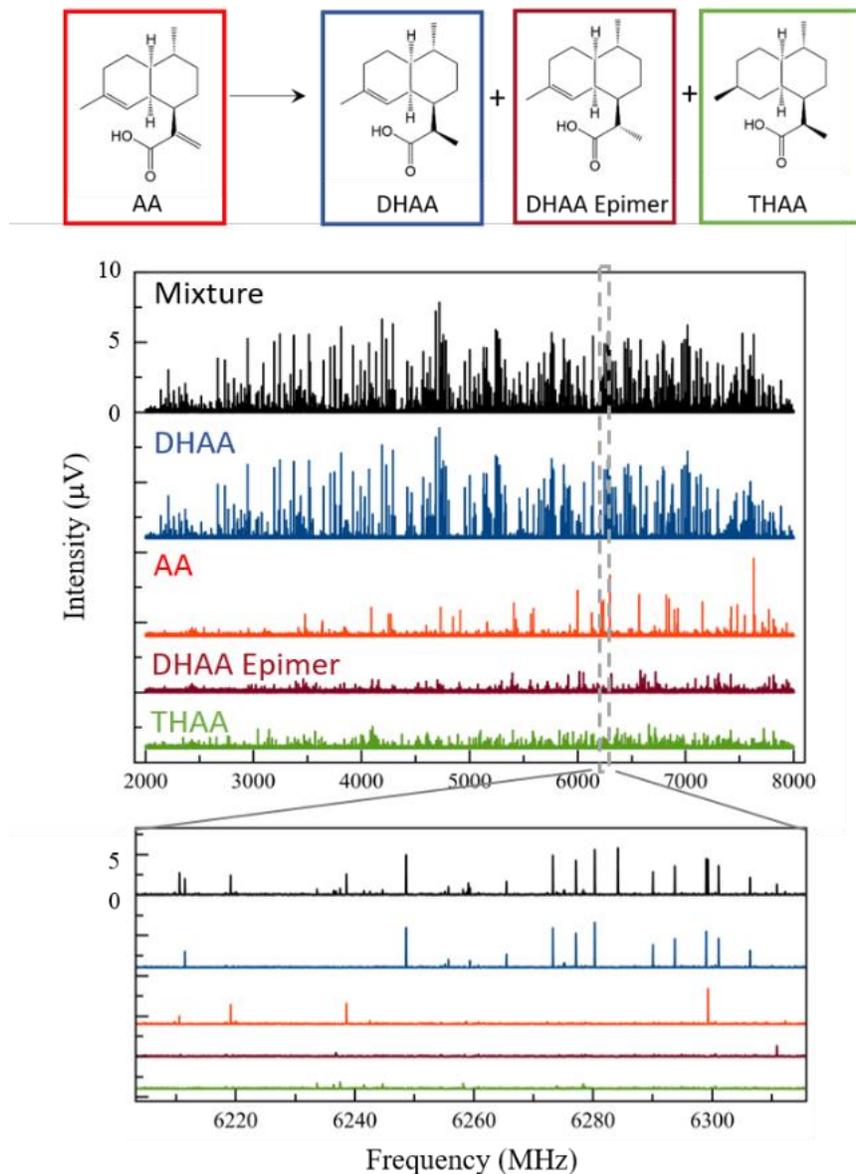
Figure 3: Broadband MRR spectrum of a crude reaction mixture from a continuous hydrogenation of artemisinic acid. The black spectrum is the full reaction mixture, while the colored traces are masked to show the individual contributions of each component (using the Hamiltonian fits to each species). The bottom panel shows an expanded view of the spectrum to show that the rotational lines of each component do not overlap with each other.

Process measurements

We next demonstrated the ability of the spectrometer to quantitatively determine the composition of a two-component mixture, using prepared mixtures of the starting material (AA) and purified product (DHAA) at five different ratios from 95:5 (DHAA:AA) to 50:50. The results of this analysis are presented in Figure 4. The instrument was operated under the same

conditions as in the process monitoring measurements, except the samples were injected via syringe from prepared solutions at each composition. The sum of the AA and DHAA concentrations was kept constant at 3 mg/mL in each sample, the same concentration as was used in the flow process.
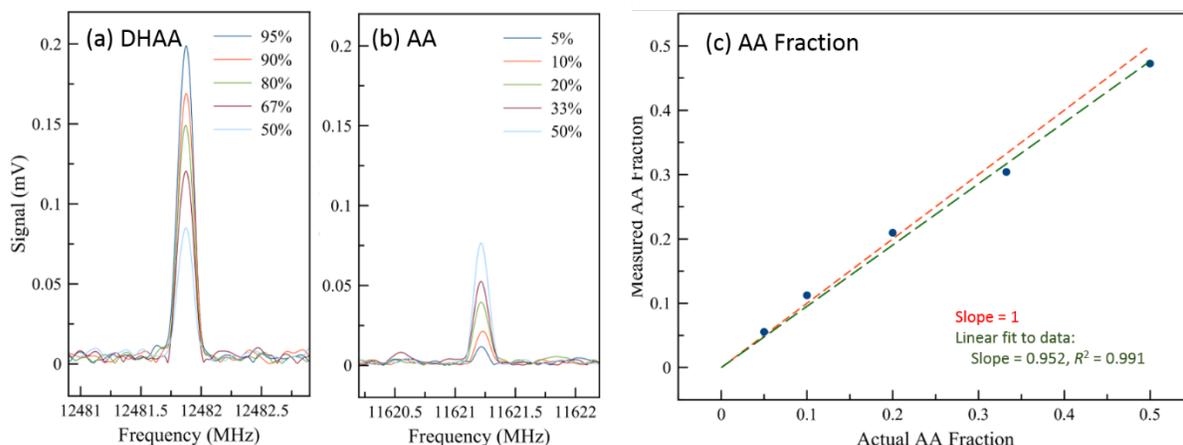

Figure 4: MRR measurements of prepared solutions of AA and DHAA, diluted to a combined concentration 3 mg/mL in ethanol. Panels (a) and (b) show the raw spectra of DHAA and AA obtained from each mixture, while panel (c) plots the measured AA fraction (defined by the ratio of the AA peak signal to the sum of the two signals) against the actual AA fraction. The overlaid lines show a slope of 1 (red) and a best fit to the data, with a fixed intercept of zero (green).

In quantifying the relative concentration of each species, we account for the conformer populations (determined directly from the broadband spectrum), calculated line strength, and the population difference between the two states measured (assuming a typical rotational temperature of 2 K). We did not correct for any possible differences in the vapor pressure of each analyte in the mixture at the measurement temperature. In this reaction, the molecular weights and structures of the different analytes are similar, and based on our observations, any vapor pressure differences appear to be small. If desired, a calibration mixture could be used for correction, if available, or benchmarking to another analytical technique.

In Figure 5 we show example data for monitoring the continuous hydrogenation of AA to DHAA. During this measurement the conditions (flow rate, temperature, and pressure) were

intentionally varied during the synthesis in order to initiate significant changes in the product distribution between samples. All four species of interest are monitored in each measurement. We note that THAA in particular, because of its saturated nature, is highly challenging for analysis by either HPLC (with UV detection) or ^{1}H NMR. MRR does not require a chromophore in the same sense as these other techniques; it requires only a permanent dipole moment, which is almost always present in chiral molecules. Therefore, MRR can help to fill analytical gaps of other techniques.
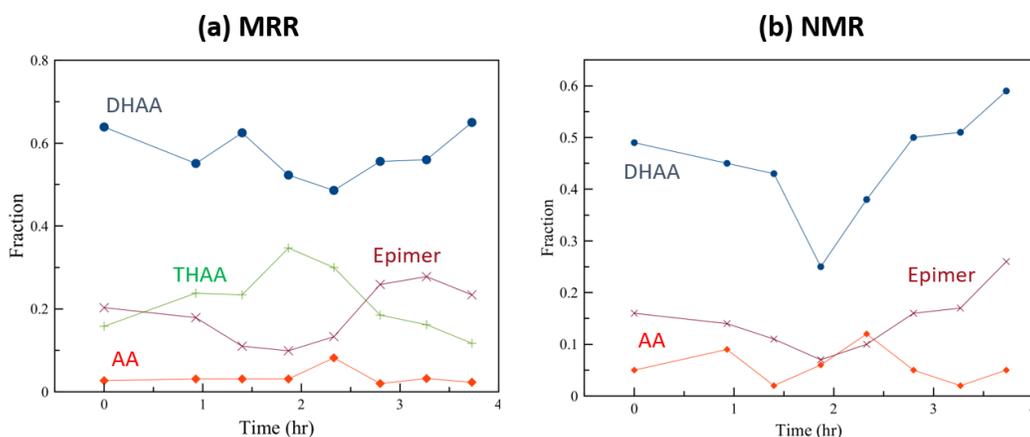

Figure 5: Left: Concentration data for a series of 8 sample measurements performed over the course of 4 hours with the MRR spectrometer connected to the synthesis flow. Right: Offline ^{1}H NMR analysis of reaction aliquots collected at the same time as the MRR analysis.

At the same time the MRR spectrometer drew a sample for analysis, a fraction of the reaction mixture was collected for comparative off-line analysis by ^{1}H NMR (Bruker 600 MHz). In these measurements, an internal standard (dimethyl sulfone) was added to determine the absolute product yield. However, due to the lack of a characteristically-shifted proton for THAA, it was not possible to determine its concentration directly. The MRR analysis instead determines the product ratios between the four relevant species. This may explain the lower overall DHAA percentage in the NMR measurements. Regardless, it can be seen that the trends in the

concentrations of the three species monitored in both samples is similar between the two analytical techniques.

Conclusion

This paper represents the first demonstration of molecular rotational resonance spectroscopy for characterization of the purity of a pharmaceutical synthetic process. The pivotal advantage of MRR over other process monitoring techniques is its high resolution and specificity: different species, including regioisomers, diastereomers, and enantiomers, can be individually resolved and quantified in a reaction mixture unambiguously. We show that the MRR technique has extremely good structural selectivity and good quantitative accuracy. Because advance sample preparation, internal standards, and calibration measurements are not needed, this represents a significant new capability for reaction monitoring in continuous synthetic processes. This method is broadly applicable to analyses of stable intermediates and products in small-molecule pharmaceutical manufacturing.

Conflict of Interest Disclosure

Authors J.L.N, M.T.M, and R.L.R are employed by BrightSpec, Inc. and have financial

interests in BrightSpec. Author B.F.G. serves on the BrightSpec Scientific Advisory Board.

Author B.H.P. has a financial interest in BrightSpec.

Supporting Information

The structures and MRR fit and calculated parameters of the species characterized in this

study are available as Supporting Information.

References

1. Carter, C.F.; Lange, H.; Ley, S.V.; Baxendale, I.R.; Wittkamp, B.; Goode, J.G.; Gaunt, N.L. ReactIR flow cell: A new analytical tool for continuous flow chemical processing. *Org. Proc. Res. & Dev.* **2010**, *14*, 393-404.
2. Moore, J.S.; Jensen, K.F. "Batch" kinetics in flow: Online IR analysis and continuous control. *Angew. Chem. Int. Ed.* **2014**, *53*, 470-473.
3. Foley, D.A.; Wang, J.; Maranzano, B.; Zell, M.T.; Marquez, B.L.; Xiang, Y.; Reid, G.L. Online NMR and HPLC as a reaction monitoring platform for pharmaceutical process development. *Anal. Chem.* **2013**, *85*, 8928-8932.
4. Foley, D.A.; Doechke, C.W.; Buser, J.Y.; Merritt, J.M.; Murphy, L.; Kissane, M.; Collins, S.G.; Maguire, A.R.; Kaerner, A. ReactNMR and ReactIR as reaction monitoring and mechanistic elucidation tools: The NCS mediated cascade reaction of a-thioamides to a-thio-b-chloroacrylamides. *J. Org. Chem.* **2011**, *76*, 9630-9640.
5. Zientek, N.; Laurain, C.; Meyer, K.; Kraume, M.; Guthausen, G.; Maiwald, M. Simultaneous ^{19}F-^{1}H medium resolution NMR spectroscopy for online reaction monitoring. *J. Mag. Reson.* **2014**, *249*, 53-62.
6. Maiwald, M.; Steinhof, O.; Sleigh, C.; Bernstein, M.; Hasse, N. Quantitative high-resolution online NMR spectroscopy in pharmaceutical reaction and process monitoring. In *NMR Spectroscopy in Pharmaceutical Analysis*, 1st Ed.; Holzgrabe, U., Wawer, I., Biehl, B., Eds; Elsevier: Oxford, **2008**; 471-491.
7. Browne, D.L.; Wright, S.; Deadman, B.J.; Dunnage, S.; Baxendale, I.R.; Turner, R.M.; Ley, S.V. Continuous flow reaction monitoring using an on-line miniature mass spectrometer. *Comm. Mass Spectrom.* **2012**, *26*, 1999-2010.
8. Zawatsky, K.; Grosser, S.; Welch, C.J. Facile kinetic profiling of chemical reactions using MISER chromatographic analysis. *Tetrahedron* **2017**, *73*, 5048-5053.
9. Welch, C.J.; Gong, X.; Cuff, J.; Dolman, S.; Nyrop, J.; Lin, F.; Rogers, H. Online analysis of flowing streams using microflow HPLC. *Org. Proc. Res. & Dev.* **2009**, *13*, 1022-1025.
10. Schafer, W.A.; Hobbs, S.; Rehm, J.; Rakestraw, D.A.; Orella, C.; McLaughlin, M.; Ge, Z.; Welch. C.J. Mobile tool for HPLC reaction monitoring. *Org. Proc. Res. & Dev.* **2007**, *11*, 870-876.

11. Malig, T.C.; Koenig, J.D.B; Situ, H.; Chehal, N.K.; Hultin, P.G.; Hein, J.E. Real-time HPLC-MS reaction progress monitoring using an automated analytical platform. *React. Chem. & Eng.* **2017**, *2*, 309-314.

12. Li, B.; Guo, W.; Chi, H.; Kimura, M.; Ramsey, E.D. Monitoring the progress of a photochemical reaction performed in supercritical fluid carbon dioxide using a continuously stirred reaction cell interfaced to on-line SFC. *Chromatographia* **2017**, *80*, 1179-1188.

13. *Guidance for Industry, PAT – A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance;* U.S. Department of Health and Human Services, Food and Drug Administration: Rockville, MD, September 2004. Available on the Web at https://www.fda.gov/downloads/drugs/guidances/ucm070305.pdf.

14. Shen, Z.; Lv, C.; Zeng, S. Significance and challenges of stereoselectivity assessing methods in drug metabolism. *J. Pharm. Anal.* **2016**, *6*, 1-10.

15. *Novel Drug Approvals for 2018.* U.S. Department of Health and Human Services, Food and Drug Administration: Rockville, MD. Accessed February 5, 2019. Available on the Web at https://www.fda.gov/drugs/developmentapprovalprocess/druginnovation/ucm592464.htm.

16. Ebinger, K.; Weller, H.N. Comparison of chromatographic techniques for diastereomer separation of a diverse set of drug-like compounds. *J. Chromatogr. A* **2013**, *1272*, 150-154.

17. Liu, W.; Gan, J.J. Separation and analysis of diastereomers and enantiomers of cypermethrin and cyfluthrin by gas chromatography. *J. Agric. Food Chem.* **2004**, *52*, 755-761.

18. Adams, R.W.; Byrne, L.; Kiraly, P.; Foroozandeh, M.; Paudel, L.; Nilsson, M.; Clayden, J.; Morris, G.A. Diastereomeric ratio determination by high sensitivity band-selective pure shift NMR spectroscopy. *Chem. Commun.* **2014**, *50*, 25122-2514.

19. Guo, C.; Shah, R.D.; Dukor, R.K.; Cao, X.; Freedman, T.B.; Nafie, L.A. Determination of enantiomeric excess in samples of chiral molecules using Fourier transform vibrational circular dichroism spectroscopy: simulation of real-time reaction monitoring. *Anal. Chem.* **2004**, *76*, 6956-6966.

20. Guo, G.; Shah, R.D.; Mills, J.; Dukor, R.K.; Cao, X.; Freedman, T.B.; Nafie, L.A. Fourier transform near-infrared vibrational circular dichroism used for on-line monitoring the epimerization of 2,2-dimethyl-1,3-dioxolane-4-methanol: A pseudo racemization reaction. *Chirality* **2006**, *18*, 775-782.

21. Mower, M.P.; Blackmond, D.G. In-situ monitoring of enantiomeric excess during a catalytic kinetic resolution. *ACS Catal.* **2018**, *8*, 5977-5982.

22. Shen, Z.; Lv, C.; Zeng, S. Significance and challenges of stereoselectivity assessing methods in drug metabolism. *J. Pharm. Anal.* **2016**, *6*, 1-10.

23. Welch, C.J. Are we approaching a speed limit for the chromatographic separation of enantiomers? *ACS Cent. Sci.* **2017**, *3*, 823-829.

24. Gordy, W.; Cook, L.R. *Microwave Molecular Spectra*, 3rd Ed. John Wiley & Sons Inc.: New York, **1984.**

25. Pate, B.H.; Evangelisti, L.; Caminati, W.; Xu, Y.; Thomas, J.; Patterson, D.; Perez, C.; Schnell, M. Quantitative chiral analysis by molecular rotational spectroscopy. In *Chiral Analysis: Advances in Spectroscopy, Chromatography, and Emerging Methods*, 2nd Ed: Polavarapu, P, Ed.; Elsevier, 2018.

26. Adamo, A.; Beingessner, R.L.; Behnam, M.; Chen, J.; Jamison, T.F.; Jensen, K.F.; Monbaliu, J.-C. M.; Myerson, A.S.; Revalor, E.M.; Snead, D.R.; Stelzer, T.; Weeranoppanant, N.; Wong, S.Y.; Zhang, P. On-demand continuous/ flow production of pharmaceuticals in a compact, reconfigurable system. *Science* **2016**, *352*, 61-67.

27. Cole, K.P.; McClary Groh, J.; Johnson, M.D.; Burcham, C.L.; Campbell, B.M.; Diseroad, W.D.; Heller, M.R.; Howell, J.R.; Kallman, N.J.; Koenig, T.M.; May, S.A.; Miller, R.D.; Mitchell, D.; Myers, D.P.; Myers, S.S.; Phillips, J.L.; Polster, C.S.; White, T.D.; Cashman, J.; Hurley, D.; Moylan, R.; Sheehan, P.; Spencer, R.D.; Desmond, K.; Desmond, P.; Gowran, J. Kilogram-scale

prexasertib monolactate monohydrate synthesis under continuous-flow CGMP conditions. *Science* **2017**, *356*, 1144-1150.

28. Korwar, S.; Amir, S.; Tosso, P.; Desai, B.K.; Kong, C.J.; Fadnis, S.; Telang, N.S.; Ahmad, S.; Roper, T.D.; Gupton, B.F. The application of a continuous Grignard reaction in the preparation of fluconazole. *Euro. J. Org. Chem.* **2017**, *2017*, 6495-6498.

29. Ro, D.-K.; Paradise, E.M.; Ouellet, M.; Fisher, K.J.; Newman, K.L.; Ndungu, J.M.; Ho, K.A.; Eachus, R.A.; Ham, T.S.; Kirby, J.; Chang, M.C.Y.; Withers, S.T.; Shiba, Y.; Sarpong, R.; Keasling, J.D. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. *Nature* **2006,** *440*, 940-943.

30. Kong, C.J.; Fisher, D.; Desai, B.K.; Yang, Y.; Ahmad, S.; Belecki, K.; Gupton, B.F. High-throughput photo-oxidations in a packed bed reactor system. *Bioorganic Med. Chem.* **2017**, *25*, 6203-6208.

31. Turconi, J.; Griolet, F.; Guevel, R.; Oddon, G.; Villa, R.; Geatti, A.; Hvala, M.; Rossen, K.; Goller, R.; Burgard, A. Semisynthetic artemisinin, the chemical path to industrial production. *Org. Proc. Res. & Dev.* **2014**, *18*, 417-422.

32. Pieber, B.; Glasnov, T.; Kappe, C.O. Continuous flow reduction of artemisinic acid utilizing multi-injection strategies—closing the gap towards a fully continuous synthesis of antimalarial drugs. *Chem. Eur. J.* **2015**, *21*, 4368-4376.

33. Feth, M.P.; Rossen, K.; Burgard, A. Pilot plant PAT approach for the diastereoselective diimide reduction of artemisinic acid. *Org. Proc. Res. & Dev.* **2013**, *17*, 282-293.

34. Brown, G.G.; Dian, B.C.; Douglass, K.O.; Geyer, S.M.; Shipman, S.T.; Pate, B.H. A broadband Fourier transform microwave spectrometer based on chirped pulse excitation. *Rev. Sci. Instrum.* **2008**, *79*, 053103.

35. Perez, C.; Lobsiger, S.; Seifert, N.A.; Zaleski, D.P.; Temelso, B.; Shields, G.C.; Kisiel, Z.; Pate, B.H. Broadband Fourier transform rotational spectroscopy for structure determination: The water heptamer. *Chem. Phys. Lett.* **2013**, *571*, 1-15.

36. Balle, T.J.; Flygare, W.H. Fabry-Perot cavity pulsed Fourier transform microwave spectrometer with a pulsed nozzle source. *Rev. Sci. Instrum.* **1981**, *52*, 33-45.

37. Suenram, R.D.; Grabow, J.-U.; Zuban, A.; Leonov, I. A portable, pulsed-molecular-beam, Fourier-transform microwave spectrometer designed for chemical analysis. *Rev. Sci. Instrum.* **1999**, *70*, 2127.